Brazilian Phlebotomines as Hosts and Vectors of Viruses, Bacteria, Fungi, Protozoa (Excluding Those Belonging to the Genus *Leishmania*) and Nematodes

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Many microorganisms—ranging from viruses, bacteria, entomophthoralic fungi, trypanosomatids and aseptate gregarines to Tylenchida and Spirurida nematodes (Warburg 1991)—have been isolated from American sand flies. Their importance as vectors of *Leishmania* species has eclipsed their role as vectors or hosts of other organisms except for Bartonellosis. In the case of trypanosomatids, studies on leishmaniasis have generated, directly or indirectly, most of the information that currently exists on these different parasites. In this chapter, we refer to findings of other monoaxenic parasites, including protozoa and bacteria, which can affect leishmanial vector efficiency. Eclectic feeding habits are responsible for epidemiological situations, in which viruses are transmitted between different vertebrates.

It is known that species such as *Bichromomyia flaviscutellata* and *Lutzomyia longipalpis* feed on both birds and mammals. However, there is evidence (Tesh et al. 1971) that some species, such as *M. micropyga*, feed on both warm- and cold-blooded vertebrates. However, so far the participation of sand flies in the transmission between different animal phyla has not been verified. If it does occur, it is most likely to be viruses because there are examples of phyla switching such as that of West Nile Virus passing form birds to man or the overwintering of the western equine encephalitis virus in snakes (Gebhardt et al. 1964).

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Viral Parasites

Vesiculoviruses (Rhabdoviridae – *Vesiculovirus* – vesicular stomatitis virus [VSV] group), phleboviruses (Bunyaviridae – *Phlebovirus* – phlebotomus fever group), and orbiviruses (Reoviridae–*Orbivirus*–Changuinola virus group) are amongst the various virus groups associated with phlebotomines in both the Old World and the New World.

It is important to note that the World Health Organization (WHO 1967) has suggested criteria for recognizing an arthropod as an arbovirus vector, which—more recently—were referenced by the International Committee on Taxonomy of Viruses (ICTV 2016). They include the following:

1. Isolation of the virus from species collected in the field;
2. Demonstration of the ability of the arthropod to become infected by feeding on a viraemic vertebrate host or an artificial suspension of the virus;
3. Demonstration of the ability of the insect to transmit biologically by stinging; and
4. Accumulation of evidence in the field, confirming the association of the arthropod with the vertebrate, in which the disease or infection is occurring.

Based on these criteria, the vectors are distributed as follows:

1. Suspected vectors (those that meet one of the above criteria);
2. Potential vectors (those that satisfy the natural infection and experimental transmission tests); and
3. Confirmed vectors (those that meet all the criteria (WHO 1967)).

From 1961 to 1995, 69 arbovirus serotypes were isolated from sand flies in several areas of the Brazilian Amazon (Fig. 1), distributed among families, genera and groups (Table 1).

Rhabdoviridae – Vesiculovirus – *VSV Group*

This genus currently consists of 16 distinct virus serotypes that have been isolated from a variety of arthropods and mammals in Asia, Africa, Europe and the Americas. Ten of them have been associated with sand flies (Comer and Tesh 1991). Six are known to cause disease in humans and domestic animals (Travassos da Rosa et al. 1984). For this reason, they are of public and veterinary health importance.

In the Brazilian Amazon region, rhabdoviruses belonging to the VSV serogroup have been frequently recovered from phlebotomine sand flies; Carajás and Marabá vesiculoviruses were isolated from pools of *Lutzomyia* spp. captured in Serra Norte, municipality of Marabá, Pará state. In addition, the Carajás virus was isolated from a pool of male *Lutzomyia* spp. This shows that vertical transmission of this agent occurs in nature (Travassos da Rosa et al. 1984a). Both agents replicate and were

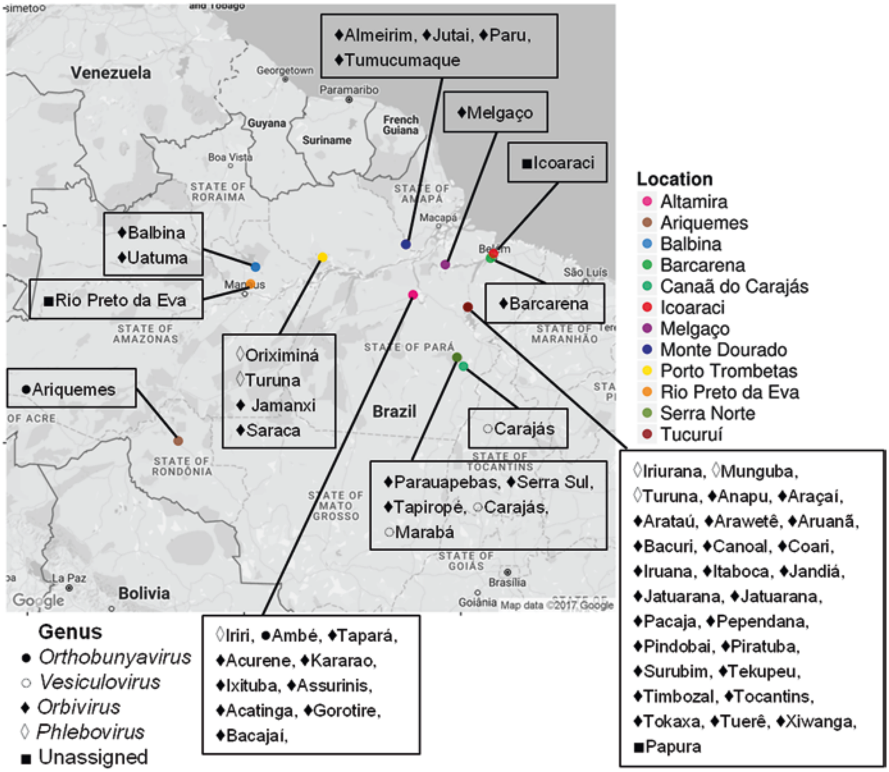

Fig. 1 Map of the Brazilian Amazonia region showing the localization of the capture sites of the sand flies from which viruses were isolated

transmitted vertically by *Lutzomyia longipalpis* after intrathoracic inoculation (Travassos da Rosa et al. 1984a). This species is presumably not the natural vector of these viruses because Carajás and Marabá came from forest areas, which are not the normal habitat of *Lu. longipalpis*. Thus, other sand-fly species are likely to serve as vectors for these viruses (Comer and Tesh 1991).

Neutralizing antibodies to Marabá virus were detected in a single human-serum sample in Brazil (Travassos da Rosa et al. 1984a). However, the source of infection of these viruses for sand flies has not been determined nor has the their potential to cause human disease.

Inhangapi is a member of the Rhabdoviridae, but it does not belong to the genus *Vesiculovirus*. This possible arbovirus was isolated only once from a pool of *L. flaviscutellata* females captured in the Catu Forest, Belém, Pará (Karabatsos 1985). in which it had been tentatively inserted. More recently, the Kairi virus, isolated from a pool of sand-fly females caught in Nova Fronteira Agrovila (km 80), Altamira, Pará, was inserted in this family due to the results obtained by electron microscopy.

Table 1 Arbovíruses isolated from Brazilian Amazonian sand flies between 1961 and 1995

Family	Genus	Antigenic group	Name	Sample	Isolation date
Rhabdoviridae	*Vesiculovirus*	VSV	Carajás	AR4113	28/01/1983
			Marabá	AR 411459	04/02/1983
Bunyaviridae	*Phlebovirus*	Phlehotomus	Ambé	AR407981	14/01/1982
			Ariquemes	AR485678	30/12/1988
			Icoaraci	AN24262	14/10/1960
			Jacundá	AR428329	31/10/1984
			Joa	AR371637	29/03/1979
			Munguba	AR389707	20/09/1980
			Oriximiná	AR385309	26–30/07/1988
			Tapará	AR413570	04/02/1983
			Turuna	AR352492	20–16/07/1978
			Uriurana	AR479776	16/12/1985
Reoviridae	*Orbivirus*	Changuinola	Acatinga	AR428250	06/09/1988
			Acurenê	AR446985	18/09/1985
			Almeirim	AR389709	20/09/1980
			Altamira	AR264277	30/07/1974
			Água Branca	AR505545	08/09/1990
			Anapu	AR496014	28–29/06/1986
			Araçai	AR425269	17–20/02/1984
			Arataú	AR428812	24/08/1984
			Arawete	AR505172	11–14/06/1987
			Aruanã	AR428815	27/08/1984
			Assurinis	Ar482249	03/09/1988
			Bacajaf	AR482267	18/09/1988
			Bacuri	AR496008	06–07/07/1986
			Balbina	AR478620	30/04/1988
			Barcarena	AR511413	18/06/1991
			Canindé	AR54342	05/04/1963
			Canoal	AR433317	01/10/1984
			Catetê	AR495605	31/10/1989
			Coari	AR433343	30/09/1984
			Gorotire	AR482251	06/09/1988
			Gurupi	AR35646	14–20/09/196 1
			Iopaka	AR491065	30/10/1984
			Ipixaia	AR490469	26/06/1986
			Iruana	AR496021	28–29/06/1986
			Itaboca	AR496034	04/07/1986
			Jamanxi	AR243090	04–12/06/1973
			Jandiá	AR440489	20/07/1985
			Jatuarana	AR440497	20/07/1985
			Jutaí	AR397374	28/05/1981
			Kararaô	AR447024	17–18/09/1985

(continued)

Table 1 (continued)

Family	Genus	Antigenic group	Name	Sample	Isolation date
			Melgaço	AR524678	/11/1993
			Ourém	AR41067	02/04/1962
			Pacajá	AR440503	20/07/1985
			Parauapebas	AR415962	20/05/1983
			Parti	AR397370	28/05/1981
			Pependana	AR440504	20/07/1985
			Pindobaí	AR482675	10–21/02/1986
			Piratuba	AR478781	14/12/1985
			Saracá	AR385278	02/07/1980
			Serra Sul	AR498935	11/05/1990
			Surubim	AR440507	20/07/1985
			Tapiropé	AR434080 2	4/10/1984
			Tekupeu	AR505169	28/05–01/06/1987
			Timbozal	AR440541	18–25/07/1985
			Tocantins	AR486776	20/05/1986
			Tocax	AR505170	02–03/06/1987
			Tuerê	AR484704	20/05/1986
			Tumucumaque	AR397956	25/05/1981
			Uatumã	AR478626	29–30/04/1988
			Uxituba	AR452652	04–20/04/1986
			Xaraira	AR490492	26/06/1986
			Xiwanga	AR505172	04–09/06/1987
Not classified	Not classified	Not grouped	Papura	AR450572	14–15/04/1985
			Rio Preto	AR540870	09–13/12/1995
Rhabdoviridae[a]	Not classified	Not grouped	Inhangapi	AR177325	28/11/1969
			Iriri	AR408005	18/02/1982
Bunyaviridae[a]	Not classified	Not grouped	Santarém	AN238758	06/06/1973

[a] Provisional family classification based on morphology and serology

Bunyaviridae – Phlebovirus – *Phlebotomus Fever Group*

Phlebotomines also serve as vectors for an appreciable number of viruses included in the genus *Phlebovirus*. Of the 39 phleboviruses currently known, most (66%) have been associated with phlebotomines. It should be noted that all isolations of Old World sand-fly viruses are from insects belonging to the genus *Phlebotomus*, whereas those from the New World are from insects belonging to American sand-fly genera. Although there are other genera, this virus–insect association undoubtedly reflects the range of vertebrate hosts of these viruses, food preferences and the geographical distribution of vectors. Phleboviruses appear to be limited to mammals, thus reflecting the preferences for mammals of the different phlebotomine genera that are their vectors (Tesh 1988).

Of the 39 recognized serotypes, 25 have been isolated in the New World (Vasconcelos et al. 2001). The prevalence of serotypes in the New World is probably a reflection of the large diversity of sand flies in the Americas. Indeed, approximately 530 different sand-fly species are currently known in the New World, whereas only approximately 370 have been reported from the Old World.

Ten phleboviruses have been associated with disease in humans, who become infected when in contact with the sand-flies' ecological niche. In the New World, this tangential mode of infection results in limited and sporadic numbers of cases, usually in people living near or in forested areas (Guerreiro et al. 1998). In addition, serological evidence of human infection has been reported with other phleboviruses, although its real potential as a disease is unknown. It is noteworthy that the diagnosis of infection by these agents is difficult and that little attention is given to such cases because they are easily confused with malaria, influenza and other viral respiratory diseases or other arbovirus diseases (Guerreiro et al. 1998). Phlebovirus Rift Valley, in addition to being a human pathogen, is also the main problem of veterinary health in Africa where small outbreaks and large epizootics have been reported for decades (Hassan et al. 2017; Peter and Morgan 1981).

In the Brazilian Amazon, 21 (9 not yet registered in the International Catalogue of arboviruses) *Phlebovirus* members have been isolated (Nunes-Neto et al. 2017; Travassos da Rosa et al. 1983). Of these, 9 were obtained from batches of female sand flies collected in various areas of the region. However, Pacui virus was also isolated from a pool of males as well as from rodents (Aitken et al. 1975). Ariquemes virus was isolated only once from a male pool, thus strongly supporting that transovarian transmission of these viruses in nature (Travassos da Rosa et al. 1998).

The other phleboviruses recorded in the region, with the exception of Itaporanga, have been isolated from naturally infected mosquitoes and vertebrates (humans and wild animals) that have not been associated with arthropods until now (Nunes-Neto et al. 2017).

It is important to note that provisionally within the family Bunyaviridae, but not included in any group or genus, is Santarém virus isolated from a rodent *Oryzomys* sp. and from a female pool of *Psychodopygus carrerai* (Travassos da Rosa et al. 1998).

Reoviridae – Orbivirus – *Changuinola Virus Group*

The Changuinola group consists of many antigenically related viruses that have been associated with sand flies, mosquitoes and wild mammals. Such viruses have been found only in tropical America (Travassos da Rosa et al. 1984).

In the Brazilian Amazon, of the 58 serotypes belonging to the Changuinola group, 52 were isolated from sand flies in several areas of the region (Table 1 and Fig. 1). Of these, only 11 are registered in the International Arbovirus Catalogue. Most of these are single isolations, which makes it difficult to understand their natural enzootic cycles. A recent study demonstrated that the orbiviruses isolated in the Brazilian Amazon are closely related with each other and constitute a highly related phylogenetic clade (Silva et al. 2014). In view of their abundance and the well-known ability of orbiviruses to form recombinants (Gorman 1979), new serotypes may continue to emerge.

Unclassified Sand-Fly Viruses

Studies are underway to identify two new possible arboviruses—Papura and Rio Preto—isolated from sand flies in Tucurui Pará and Rio Preto da Eva, Amazonas, respectively (Travassos da Rosa et al. 1998) Rio Preto is a orthobunyavirus, whereas Papura virus has yet to be defined as to taxonomic status and thus remains as new ungrouped and unclassified virus.

Summary

The diversity of virus types indicates the biological complexity of these agents and suggests that the potential vector role as well as that the public-health importance of sand flies has not been sufficiently appreciated. However, viral infection of male sand flies indicates that the transovarian transmission of certain viruses associated with these insects occurs naturally and that the ecology of some of these agents is quite different from those of mosquito-borne viruses.

Four phleboviruses (Alenquer, Candiru, Morumbi and Serra Norte) were found in febrile Brazilian patients; however, to date they have not been recorded in sand flies. Sloths are a known food source for sand flies, and infections of Changuinola and phlebovirus complexes have already been reported from them, but these viruses have not been found in sand flies. This dissociation of the isolates of insects and mammals makes one wonder whether they are in fact transmitted by sand flies.

Bacterial Parasites

In 1964 Brooks (1964) drew attention to the potential importance of intestinal symbionts to insects of medical importance, but phlebotomines were not mentioned. Worldwide there are very few studies on the bacterial fauna of sand-fly intestines and the first of Brazilian sand flies was that of Oliveira et al. in 2000 (Oliveira et al. 2000) who made cultures from the guts of female *Lu. longipalpis* captured in the Lapinha cave in Minas Gerais. This, together with other studies using the same methods (Pereira de Oliveira et al. 2001; Gouveia et al. 2008) and two using metagenomic analyses (McCarthy et al. 2011; Sant'Anna et al. 2012), revealed the presence of 52 different bacteria (Table 2) in the intestinal tracts of Brazilian sand flies of the *Lu. longipalpis* complex. The drawback with culturing is that many bacteria go undetected because they do not grow in culture. In a more recent publication, Kelly et al. (2017) identified 609 microorganism OTUs in a 16S metagenomic analysis of the midgut of laboratory-bred *Lu. longipalpis.* Many of those listed in Table 2, such as Serratia and Enterobacteria, were found; however, of the 121 identified to species only a few coincided with those found previously (Table 2). What is surprising is that species of *Acanthamoeba* and *Wolbachia* were identified. This suggests that other material other than those of the actual midgut contents were inadvertently included in the analysis.

Three important questions exist: Where do the bacteria come from? WHAT is their effect on the fly? How does this effect their vectorial capacity? It seems logical that they are ingested principally in the larval stage and, to a lesser degree, in the adult stage. The larvae feed on decaying material of vegetable and animal origin so they can become infected with both plant and animal pathogens. Some of these may be beneficial to the fly. For instance, the presence of nitrogen-fixing bacteria of the genus *Bradyrhizobium* could help them fix nitrogen. However, pathogenic bacteria could reduce the fly's vigour and longevity. In addition, bacteria

Table 2 Bacteria found in the intestine of Brazilian *Lu. longipalpis* and *Lu. cruzi*

Group/family	Genus/species	Source	*long.*	*cruzi*
Moraxellaceae	*Acinetobacter baumannii*	2,3,4	+	–
Moraxellaceae	*A. bucaniid*	4	+[a]	–
Moraxellaceae	*Acinetobacter* sp.	5	+	–
Moraxellaceae	*A. lwoffi*	1.2	+	–
Bacillaceae	*Anoxybacillus flavithermus*	4	+	–
α Proteobacterium	*Asaia* sp.	6	+	–
Bacillaceae	*Bacillus thuringiensis*	1	+	–
Alphaproteobacteria	*Bradyrhizobium japonicum*	5	+	+
Burkholderiaceae	*Burkholderia cepacia*	2,3	+	–
Alphaproteobacteria	*Caulobacter* sp.	5	+	+
Bacteroidetes	*Chryseobacterium meningosepticum*	5	–	+
Enterobacteriaceae	*Citrobacter freundii*	3	+	–
Firmicutes	*Clostridium disporicum*	5	+	+
Firmicutes	*Clostridium* sp.	5	+	–
Firmicutes	*C. glycolicum*	5	–	+
Enterobacteriaceae	*Enterobacter aerogenes*	2,3	+	–
Enterobacteriaceae	*E. amnigenus*	3	+	–
Enterobacteriaceae	*E. cloacae*	1,2,3	+	–
Enterobacteriaceae	*E. gergoviae*	2,3	+	–
Enterobacteriaceae	*E. sakazakii*	2	+	–

(continued)

Table 2 (continued)

Group/family	Genus/species	Source	*long.*	*cruzi*
Enterobacteriaceae	*E. taylorae*	3	+	−
Gammaproteobacteria	*Erwinia billingiae*	5	+	−
Enterobacteriaceae.	*Escherichia coli*	3	+	−
Pseudomonadaceae	*Flavimonas orizihabitans*	1,3	+	−
Bacillaceae	*Geobacillus kaustophilus*	4	+	−
Enterobacteriaceae	*K. oxytoca*	3	+	−
Enterobacteriaceae	*K. ozaenae*	3	+	−
Enterobacteriaceae	*K. pneumoniae*	5	+	−
Firmicutes	*Lactobacillus zymae*	5	−	+
Microbacteriaceae	*Leifsonia xyli*	4	+[a]	−
Betaproteobacteria	*Leptothrix* sp.	5	+	−
Enterobacteriaceae	*Morganella morganii*	3	+	−
Actinobacteridae	*Nocardioides albus*	5	+	−
Enterobacteriaceae.	*Pantoea agglomerans*	3	+	−
Propionibacteriaceae	*Propionibacterium acnes*	4,5	+	−
Proteobacterium	(uncultured)	4	+[a]	−
Pseudomonadaceae	*Pseudomonas aeruginosa*	2,3	+	−
Pseudomonadaceae	*P. fluorescens*	2	+	_
Pseudomonadaceae	*P. putida*	1,3	+	−
Ralstoniaceae	*Ralstonia* sp.	5	+	+
Ralstoniaceae	*Ralstonia pickettii*	4	+	−
Enterobacteriaceae	*Serratia liquefaciens*	3	+	−
Enterobacteriaceae	*S. marcescens*	2,3	+	−
Enterobacteriaceae	*Serratia* sp.	5	+	−
Bacteroidetes	*Sphingobacterium daejeonense*	5	+	−
Firmicutes; Bacillales	*Staphylococcus* sp.	3	+	−
Firmicutes; Bacillales	*S. xylosus*	5	−	+
Streptomycetaceae	*Streptomyces coelicolor*	4	−	−
Xanthomonadaceae	*Stenotrophomonas maltophilia*	1,2,3	+	−
Veillonellaceae	*Veillonella* sp.	4	+[a]	−
Flavobacteriaceae	*Weeksella virosa*	3	+	−
Enterobacteriaceae	*Yokenella regensburgei*	2	+	−

Source: 1 = Oliveira et al. (2000); 2 = Pereira de Oliveira et al. (2001); 3 = Gouveia et al. (2008); 4 = McCarthy et al. (2011); 5 = Sant'Anna et al. (2012); 6 = Sant'Anna et al. (2014)

[a] Only found in males

of medical importance have been found in sand flies so could they be vectors of these pathogens to man and other animals?

Research on sand fly−gut bacteria is in its infancy. *Bacillus thuringiensis* could be used to control sand-fly populations (Yuval and Warburg 1989), but would this produce unforeseen problems? In addition, it is intriguing and challenging to see if the manipulation of this micro fauna influences vectorial capacity. Adler and Theodor (1927) suggested that bacterial competition could modulate the infections of leishmania in the sand fly. Over the years evidence has been growing to vindicate this opinion. Recent papers (Sant'Anna et al. 2014; Kelly et al. 2017) investigating the interaction between *Leishmania* and the bacterial gut fauna of experimental infections in *Lu. longipalpis* indicated that bacteria can affect the parasite's development positively or negatively. It appears that bacteria facilitate metacyclogenesis. Besides this the level of regurgitated during feeding plays an important role in the immunological response of the host. Their presence in the initial lesion leads to the recruitment of cells that facilitate the parasite's expansion and consequently future pathologies (Kelly et al. 2017). When *Leishmania*-infected flies were treated with antibiotics, parasite multiplication and the development of metacyclic promastigotes was reduced (Wilson et al. 2017). However, not all bacteria are good for *Leishmania*. Sant'Anna et al. (2014) found that *Serratia marcescens* inhibited leishmanial development but that *Lu. longipalpis* infected with *Leishmania* lived longer than those infected with just *Serratia*. Thus, evidence is accumulating suggesting that the beneficial or detrimental effect of gut bacteria on *Leishmania* development is related to the species of bacteria. In the case of infections of pathogenic bacteria, the sand fly is protected by the *Leishmania*.

The epidemiological implications of these recent findings of the pivotal importance of the intestinal microbiome are extremely important. Could the bacterial fauna acquired by the immature stages or the adults feeding on plants increase or decrease vectorial capacity? McCarthy et al. (2011) compared the microbiota of *Lu. longipalpis* captured in an urban area of Argentina with those captured in a wild environment in Minas Gerais. They found that the bacterial microbiota of the two populations was completely different. Only one bacterium was found in the urban flies compared with nine in the wild flies. In a set of experiments, Sant'Anna et al. (2014) showed that when pre-feeding laboratory-reared *Lu. longipalpis* with *Asaia*—or with the yeast *Pseudozyma* together with *Asaia* or *Ochrobactrum* intermedium—both diminished the midgut promastigote population. *O. intermedium* has so far only been found in laboratory *Lu. longipalpis* colonies.

Information of the effects of enteric bacterial entomopathogens in Brazilian sand flies is limited to *Lu. longipalpis* and *Leishmania*; however, it may apply to infections with other trypanosomatids. Sant'Anna et al. (2014) concluded that populations that are susceptible to *Leishmania* infection may survive bacterial enteric pathogens better than those that are resistant to leishmanial infection. This implies that *Leishmania* circulating in an enzootic or endemic environment will favour the expansion of a susceptible vector population.

Fungi

Pathogenic fungi of insects belong to the order Entomophthorales, which consists of five families (Keller and Petrini 2005). Fungi can be a problem in colonies where they may form hyphal mats, in which the larvae become entangled and eventually die. However, they are rarely found in wild-caught flies: The first record is that of Warburg (1991) who noted mycelia in the thoracic muscle of a female *Pintomyia pia* collected in Colombia. The parasite was considered to belong to the genera *Entomophthora* or *Conidiobolus*. The only records we could find of entomophthorales in Brazilian sand flies were those of McCarthy et al. (2011). In their metagenomic analysis they identified *Peronospora conglomerata*, *Cunninghamella bertholletiae*, *Mortierella verticillate* and *Toxicocladosporium irritans* in *Lu. longipalpis* from the Lapinha cave.

Microsporidia

For many years microsporidia were considered protozoa, but they are now accepted as being a sister group to the fungi. They are present in all the major animal groups and are common in insects. Under the light microscope, these infections appear as off white- to white-coloured spots. Phylogenies using ribosomal DNA indicate that morphology is unreliable for genus identification (Brown and Adamson 2006).

Family Pleistophoridae Stempell, 1909

The assignment of the genera *Pleistophora* and *Vavraia* to a family is controversial. They have both been placed in the family Glugeidae, and *Vavraia* has been designated as the type species of the family Thelohaniidae. However, for the moment we follow Sprague et al. (1992) who considered them both to be in the family Pleistophoridae. These controversial issues will undoubtedly be resolved by future molecular studies.

The first infection of a microsporidian in a Brazilian sand fly was registered by Ward and Killick-Kendrick (1974) in the midgut of a female *Ps. lainsoni* collected at km 46 of the Altamira–Itaituba section of the Transmazônica Highway. Subsequently (Lainson et al. 1977), two infections were found in the Malpighian tubules and two in the midgut of *Ny. umbratilis*. Those from the Malpighian tubules were considered to belong to the genus *Pleistophora* due to the morphology of the sporoblasts that appeared in the form of a bunch of grapes. One from the mid-gut was classified as belonging to the family Thelohaniidae due to schizonts with diplokaryon nuclei and octonucleate sporonts. The second midgut parasite was composed of predominantly uninucleate spores often in chains. It was tentatively placed in the genus *Microsporidium*. A *Thelohania*-like parasite has also been recorded in *Ps. maripaensis* (Canning 1977).

Matos et al. (2006) studied the infection of a microsporidian that they found in a *Lu. longipalpis* population close to the city of Teresina, Piaui. The infection was found in the subcuticular tissue of the larval and pupal abdomen and in the

Malpighian tubules and midgut of the adult flies. These investigators described the morphology and ultrastructure of the parasite, considered is to be a new species belonging to the genus *Vavraia*, and named it *Vavraia lutzomyiae*. This is the first named species of a microsporidian from a Brazilian sand fly. It seems very unlikely that the microsporidia recorded from Amazonian sand flies in the 1970s all belong to the same species.

Protozoal Parasites

Family Lecudinidae Mingazzini, 1891 – Aseptate Gregarines

Aseptic gregarines (Eugregarinorida, Aseptatorina and Lecudinidae) of sand flies were initially classified as belonging to the genus *Monocystis*. Later, they were transferred to the genus *Ascocystis* (Scorza & Carnevali 1981). Because this name was preoccupied, the genus *Ascogregarina* was created to replace it. However, analyses of the small-subunit rRNA-gene sequences by Votypka et al. (2009) showed that the sand-fly gregarines are very different from those of mosquitoes, which are classified as *Ascogregarina.* Because of this Votypka et al. (2009) created the genus *Psychodiella* for the sand-fly gregarines. Thus, the valid name for the gregarines of Brazilian sand flies is *Psychodiella chagasi.*

Gregarine infections (*Monocystis mackiei*) were first described in 1927 (Shortt and Swaminath 1927) in Indian specimens of *Phlebotomus argentipes*. However, it was only in 1961 that Adler and Mayrink (1961) found similar parasites in *Lu. longipalpis* from Minas Gerais, Brazil. About 20% of *Lu. longipalpis*, both wild and laboratory-bred, were infected, and the parasite was considered a new species, *Monocystis chagasi* (Adler and Mayrink 1961). Gregarines were subsequently recorded in *Bi. flaviscutellata* (Lewis et al. 1970) *Ev. Evandroi, Ev. Sallesi* and *Ps. complexus.* Due to their morphological similarities to the parasite described by Adler and Mayrink, they were all considered to be *P. chagasi*.

It is debatable as to whether the aseptate gregarines infections found in the different species of Brazilian sand flies are in fact *P. chagasi*. Detailed morphological studies of the sporocysts were not made. In addition, there is also evidence of host specificity. Wu and Tesh (1989) noted that *P. chagasi* infected large numbers of Old and New World sand flies, but its complete cycle only occurred in *Lu. longipalpis*. Molecular studies are needed to elucidate the specific status of the *Psychodiella* infections of different Brazilian sand flies.

The life cycle is simple. Two morphologically similar trophozoites are confined to a cyst. The two become gamonts that sprout and divide to produce gametocytes. These fuse together to form a zygote, the walls of which thicken to give rise to the typical sporocyst. It adheres to the surface of the egg, and the larva becomes contaminated when it hatches. Although infections are frequently severe and result in the enlargement of accessory glands, there is no evidence yet of any effect on fertility or egg production. However, in experimental infections of *P. chagasi* in *Lu. longipalpis*, Wu and Tesh (1989) noted that, although fertility was not affected, there was a significant reduction in adult longevity.

Family Trypanosomatidae Doflein, 1901

Finding trypanosomatids in Brazilian sand flies is mostly a by-product of leishmaniases epidemiological studies. A list of 66 of these infections is given in Table 3 based on the information contained in 26 papers (Arias et al. 1985; Brazil and Ryan 1984; de Souza et al. 2017; Ferreira et al. 2008, 2015; Freitas et al. 2002; Galati et al. 2006; Gil et al. 2003; Hoch et al. 1986; Lainson and Shaw 1979; Lainson et al. 1973; Naiff et al. 1989; Pessoa et al. 2007; Rogers et al. 1988; Ryan et al. 1987a, 1987b; Shaw 1992; Shaw and Lainson 1972; Shaw et al. 1987; Sherlock and Pessôa 1966; Silveira et al. 1991; Souza et al. 1998; Teixeira et al. 2011; Viola et al. 2008; Williams and Coelho 1978; Ryan et al. 1987a). Few have been isolated and characterized. Some have been listed as belonging to the genera *Crithidia*, *Blastocrithidia, Strigomonas, Endotrypanum* and *Trypanosoma*; however, this characterization should be viewed with reservation because in some cases it is based on morphology. Camargo (1999), when discussing the morphology of trypanosomatids, drew attention to the fact that it is not a reliable character to distinguish genera of trypanosomatids found in plants and insects.

The scarcity of knowledge about dietary preferences of sand flies makes it almost impossible to associate infections with trypanosome species. However, there is strong evidence that Brazilian sand flies are vectors of trypanosomes of mammals, lizards, snakes and frogs. Evidence is slowly accumulating suggesting that certain genera are linked to the transmission of trypanosomes of the different vertebrate phyla, which reflects their host preferences. Potentially there is a danger that fewer flagellates, other than *Leishmania*, will be recorded in the future because present-day molecular methods used to detect *Leishmania* do not detect trypanosomes or monoxenous parasites.

Monoxenous Trypanosomatids

The first confirmed finding of a monoxenous parasite in sand flies is that of Wallace and Hertig (1968). Based on ultrastructural studies of the kinetoplast of a flagellate isolated from a Panamanian phlebotomine, *Lu. sanguinaria*, they considered that the parasite belonged to the genus *Crithidia*. To date there are few records of suspected or confirmed monoxenous flagellates in Brazilian sand flies compared to with those of *Leishmania*.

Table 3 Infections of Brazilian sand flies by different trypanosomatid genera

Sand fly species	Parasite[a]	State	Literature source
Evandromyia edwardsi	*Trypanosoma* sp.	MG	Williams and Coelho (1978)
E. evandroi	*Trypanosoma* sp.	MA	Brazil and Ryan (1984)
E. evandroi	*Trypanosoma* sp.	DF	Ferreira et al. (2015)
E. infraspinosa	*Trypanosoma* sp.	PA	Shaw and Lainson (1972)
E. infraspinosa	*Trypanosoma* sp. (An03)	RO	Gil et al. (2003), Ferreira et al. (2008)
E. inpai	Trypanosomatid[b]	PA	Ryan et al. (1987b)
E. pinottii	*Trypanosoma* sp.	PA	Ryan et al. (1987b)
E. sallesi	*Trypanosoma* sp.	MG	Williams and Coelho (1978)
E. saulensis	*Trypanosoma* sp.	PA	Shaw and Lainson (1972)
Lutzomyia gomezi	*Trypanosoma* sp.	RO	Gil et al. (2003), Ferreira et al. (2008)
L. almerioi	*Strigomonas galati*	MS	Galati et al. (2006), Teixeira et al. (2011)
M. cayennensis	*Trypanosoma* sp.	AM	Ryan et al. (1987a)
M. micropyga	*Trypanosoma* sp.	BA	Sherlock and Pessôa (1966)
M. rorotaensis	*T. thecodactylus*	PA	Lainson and Shaw (1979)
M. rorotaensis	*Trypanosoma* sp.	PA	Arias et al. (1985)
M. rorotaensis	*Trypanosoma* sp.	PA	Arias et al. (1985)
M. rorataensis	Trypanosomatid[b]	PA	Ryan et al. (1987b)
M. rorataensis	Trypanosomatid[b]	RO	Souza et al.(1998)
M. rorataensis	Trypanosomatid[b]	AM	Pessoa et al. (2007)
M. trinidadensis	*Trypanosoma* sp.	PA	Ryan et al. (1987a, 1987b)
Migonemyia migonei	Monoxenous parasite	AP	de Souza et al. (2017)
Nyssomyia anduzei	*Endotiypanum* sp.	PA	Shaw (1992)
Ny. anduzei	*Endotrypanum* sp.[c]	AM	Rogers et al. (1988)
N. antunes	Trypanosomatid[b]	PA	Lainson and Shaw (1979)
N. antunesi	*Trypanosoma* sp.	PA	Silveira et al. (1991)
N. umbratilis	*Endotrypanum* sp.[c]	AM	Rogers et al. (1988)
N. umbratilis	Trypanosomatid[b]	PA	Ryan et al. (1987a)
N. umbratilis	Trypanosomatid[b]	PA	Ryan et al. (1987a)
N. umbratilis	*Trypanosoma*?	AP	Freitas et al. (2002)
N. whitmani	*Trypanosoma* sp.	BA	Hoch et al. (1986)
N. whitmani	*Blastocrithidia*	DF	Ferreira et al. (2015)
*P. damascenoi**	*Trypanosoma* sp	AM	Arias et al. (1985)
P. nevesi	trypanosomatid[b]	PA	Arias et al. (1985)
P. neves	Trypanosomatid[b]	AC	Arias et al. (1985)
P. nevesi	Trypanosomatid[b]	RO	Arias et al. (1985)
P. dendrophila	Trypanosomatid[b]	PA	Lainson and Shaw (1979)
P. dendrophila	Trypanosomatid[b]	PA	Ryan et al. (1987a, 1987b)
P. dendrophila	*Trypanosoma* sp. (An03)	RO	Gil et al. (2003), Ferreira et al. (2008)
P. dendrophila	*Endotrypanum*?	AP	Freitas et al. (2002)
P. (shannoni series) sp	*Endotrypanum* sp.[d]	PA	Arias et al. (1985)

(continued)

Table 3 (continued)

Sand fly species	Parasite[a]	State	Literature source
P. (shannoni series) sp	*E.schaudinni*[d]	RO	Arias et al. (1985)
P. (shannoni series) sp	*Endotrypanum* sp.	RO	Arias et al. (1985)
P. (shannoni series) sp	*T. rangeli*[d]	RO	Arias et al. (1985)
P. (shannoni series) sp	Trypanosomatid[b]	AM	Arias et al. (1985)
P. (shannoni series) sp	Trypanosomatid[b]	AC	Arias et al. (1985)
*P. bigeniculata***	*Endotrypanum* sp.[c]	AM	Rogers et al. (1988)
*P. bigeniculata***	Trypanosomatid[b]	AM	Pessoa et al. (2007)
Sciopemyia fluviatalis	Trypanosomatid[b]	PA	Ryan et al. (1987b)
S. servulolimae	*Trypanosoma* sp.(An03)	RO	Gil et al. (2003), Ferreira et al. (2008)
*S. sordellii****	*Trypanosoma* sp	PA	Ryan et al. (1987a)
*S. sordellii****	*Trypanosoma* sp.	PA	Silveira et al. (1991)
S. sordellii	*Trypanosoma* sp.	PA	de Souza et al. (2016)
S. sordellii	*Trypanosoma* sp.(An03)	RO	Gil et al. (2003), Ferreira et al. (2008)
S. sp.	*Trypanosoma* sp.(An03)	RO	Gil et al. (2003), Ferreira et al. (2008)
Psychodopygus amazonensis	Typanosomatid[b]	PA	Lainson et al. (1973)
P. amazonensis	Trypanosomatid[b]	PA	Ryan et al. (1987b)
P. ayrozai	Trypanosomatid[b]	BA	Hoch et al. (1986)
P. clausteri	trypanosomatid[b]	PA	Ryan et al. (1987a)
P. clausteri	*T. freitasi*	AM	Naiff et al. (1989)
P. "complexus"	Trypanosomatid[b]	PA	Ryan et al. (1987b)
P. davisi	*Trypanosoma* sp.	PA	Shaw and Lainson (1972)
P. davisi	Trypanosomatid[b]	PA	Ryan et al. (1987a, 1987b)
P. davisi	*Trypanosoma* sp.	BA	Hoch et al. (1986)
P. paraensis	Trypanosomatid[b]	PA	Ryan et al. (1987a)
P."wel1comei"	Monoxenous parasite[e]	PA	Ryan et al. (1987a), Shaw et al. (1987)
Viannamyia tuberculata	*Trypanosoma* sp.[f]	RO	Viola et al. (2008)

Names that are in the original paper: [*]*Lutzomyia spinose*, [**]*Lutzomyia shannoni*, and [***]*Lutzomyia nordestina*

Species names in quotes indicate the most likely identification based on males captured at the same time

An03 = An anuran trypanosome clade

[a] Identifications are based mainly on morphology

[b] The authors do not refer to genus except that it is not a *Leishmania*

[c] Based on kinetoplast DNA probes

[d] Based on isoenzyme analysis

[e] Based on the presence of choanomastigotes

[f] Based on V7V8 SSU rDNA the isolate was similar to snake trypanosomes

[g] Unpublished observation quoted in Williams and Coelho (1978)

Flagellates that grew well in culture were found in female *Psychodopygus wellcomei* from Pará State (Ryan et al. 1987a; Shaw et al. 1987) and a female *Migonemyia migonei* from Amapá State (de Souza et al. 2017). They did not react with any of the leishmania-specific monoclonal antibodies and morphologically resembled insect parasites. Because of this and their morphology, they were tentatively considered to be Crithidia. Using primers that amplified the D7 region of the 24Sα rRNA gene Ferreira et al. (2015, no. 5358) identified a *Blastocrithidia* species in a female *Ny. whitmani* captured in a rural peri-domiciliary location of the Federal District.

During epidemiological studies of the phlebotomine fauna of forested areas of the Serra da Bodoquena, Mato Grosso do Sul State, Galati et al. (2006) isolated a flagellate from female *Lu. almerioi* that again did not react with any leishmania-specific monoclonal antibodies. This same isolate was studied by Teixeira et al. (2011) in their review of the monoxenous genera *Angomonas* and *Strigomonas*. They concluded that it was a new species and named it *Strigomonas galati* together with its symbiont, which received the name *Kinetoplastibacterium galatii*. This isolate is the first monoxenous parasite of Brazilian sand flies to receive a specific name.

The negative reaction with leishmanial-specific monoclonal antibodies seen with parasites of the Amazonian sand flies was the same as that seen with *S. galati,* which adds weight to the conclusion that they were monoxenous flagellates. However, it is debatable as to whether they belonged to the genus *Crithidia* or to one of the symbiont-bearing genera such as *Angomonas* or *Strigomonas.* Such questions can best be answered using molecular phylogenetic markers.

Thus far, there are few records of monoxenous trypanosomatids in sand flies. Wallace and Hertig (1968) suggested that the parasite they found may not be specific for sand flies and could represent an accidental infection. Another possibility is that some monoxenous parasites of dipterans are opportunistic and infect a wide variety of different hosts, which would ensure their survival.

Trypanosomes of Amphibia

The first association of sand flies with anuran trypanosomes in the Americas was that of Anderson and Ayala (1968) who experimentally infected *M. vexator* with *Trypanosoma bufophlebotomi* that had fed on California Toads (*Anaxyrus boreas halophilus*). Ferreira et al. (2008) showed that trypanosomes of Brazilian frogs and toads fell into three clades. Clade An01 were trypanosomes of hylids, and clades An02 and An03 harboured trypanosomes of toads and frogs. A fourth clade, An04, was composed of trypanosomes from African, European and North American anurans.

An03 appears to be a lineage confined to Amazonian nocturnal frogs and toads that occupy the same terrestrial habitats and aquatic breeding sites. Trypanosomes were identified in *Evandromyia infraspinosa* from Pará and Rondônia (Shaw and Lainson 1972; Gil et al. 2003); *Psathyromyia dendrophila* from Pará and Rondônia (Gil et al. 2003; Lainson and Shaw 1979; Ryan et al. 1987a, 1987b), *Sciopemyia* sp. (Gil et al. 2003), *Sc. fluviatalis* (Ryan et al. 1987b), *Sc. servulolimae* from Rondônia

(Gil et al. 2003) and *Sc. sordellii* from Pará and Rondônia (de Souza et al. 2016; Ryan et al. 1987a; Silveira et al. 1991). The anuran clade An03 was identified in the following species from Rondônia: *Ev. infraspinosa, Ps. dendrophila, Sciopemyia sp., Sc. Servulolimae* and *Sc. sordellii*. These results strongly suggest that the evandromyias and sciopemyias are important vectors of anuran trypanosomes in Amazonia. It is difficult to know the importance of *Ps. dendrophila* as a vector of anuran trypanosomes. This species appears to be a catholic feeder and has been found infected with mammalian flagellates such as *Endotrypanum.* However, the *Psathyromyia* (Shannoni series), to which this species belongs, contains 19 species that are morphologically similar and can be confused. For instance, both *Ps. dendrophyla* and *Ps. shannoni* have been recorded from the same regions, and their feeding habits are not well defined.

Trypanosomes of Lizards

Christensen and Telford (1972) found a trypanosome, *Trypanosoma thecadactyli*, in a Panamanian forest gecko (*Thecadactylus rapicaudus*) that was experimentally infected *M. trinidadensis*. Both the lizard and sand fly are found from Mexico to Brazil. In the Jari river region of northern Pará, Lainson and Shaw (1979) found flagellates in *Mi. rorotaensis* that had fed on *T. rapicaudus*, which strongly suggested that they belonged to *T. thecadactyli*. Sherlock sand Pessôa (1966) suggested that flagellates found in *Mi. micropyga* captured in animal holes or on tree trunks belonged to a lizard *Leishmania*. This statement seems incorrect because the parasites in their illustrations are like trypanosomes. The close association between sand flies that inhabit tree trunks and lizards suggests that they are the natural vectors of this reptilian trypanosome. In an analysis of three *Mi. micropyga*, two reacted with cold-blooded animal antisera (reptiles and amphibians) and one with mammalian antisera (Tesh et al. 1971). This adds weight to the hypothesis that *M.* species are the vectors of lizard trypanosomes in Brazil.

Trypanosomes of Mammals

There is good circumstantial evidence suggesting that neotropical phlebotomines are the vectors of some *T. (Megatrypanum)* trypanosomes of bats and rodents. In Peru, Herrer (1942) observed that *T. phyllotis* of cricetid rodents of the genus *Phyllotis* developed in the intestine of *Lu. noguchii* and that specimens of this same sand fly collected in rodent nests were infected. In Costa Rica, Zeledon and Rosabal (1969) observed that the trypanosome *T. leonidasdeanei* of insectivorous bats developed in *Dampfomyia vespertilionis.* This phlebotomine is found in tree hollows inhabited by bats. Christensen and Herrer (1975) considered it as a vector suggesting, as had Hoare (1972), that transmission would occur by the ingestion of infected sand flies.

Naiff et al. (1989), working in Amazonas State, found a natural infection of *T. freitasi* in *Psychodopygus clausteri* suggesting it as its vector. This marsupial trypanosome is morphologically similar to *T. phylloti.* Previously, Professor Leonidas Deane (1964) had tried unsuccessfully to experimentally infect triatomines with this same trypanosome, adding weight to the hypothesis that these megatrypanums are transmitted by sand flies.

The infection by *T. rangeli* in sand flies of the Shannoni series from Rondônia is a good example of the difficulties in interpreting the exact meaning of an infection (Arias et al. 1985). Trypanosomes of this group are transmitted by reduviids, and there is no evidence that phlebotomines could be the vectors. This single record was based on a culture made from a fly that had consumed a residual blood meal. This underscores the fact that merely finding a trypanosome in any hematophagous insect should be interpreted with caution, especially if blood is present in the intestine.

Parasites of the Genus *Endotrypanum*

Most of these parasites were found while searching for *Leishmania* infections. It is quite possible that some of infections attributed as being *Leishmania* were in fact *Endotrypanum*. The reservoirs of these parasites are principally sloths, especially two-toed sloths of the genus *Choloepus*, which is also an important reservoir of numerous species of Latin American *Leishmania*. In Pará State, of 33 *Endotrypanum* infections 16 were mixed *Endotrypanum*/*Leishmania*. However, up until now no mixed infections of these two parasites have been found in phlebotomines that are known to feed on sloths such as *Ny. anduzei, Ny. umbratilis* and *Psathyromyia bigeniculata* (Lainson et al. 1981a; Lainson et al. 1981b). Such findings may in part be linked to the methods of isolation employed, which act as septic filters. For example, if sand-fly material is inoculated into hamsters, *Leishmania* may be isolated, but *Endotrypanum* will not because this flagellate does not infect laboratory animals.

However, two other factors can influence mixed infections: There may be unknown levels of specificity of both parasites relative to the host, or one parasite could inhibit the development of the other (Pacheco et al. 1987). Both options are open to experimentation, but to date there are no theoretically reasons for believing that phlebotomine cannot be infected by both parasites. In practice, this observation has never been made, but with DNA-specific oligonucleotides and PCR techniques infections from primary isolates can now be examined in the search for mixed infections.

The first indication that *Endotrypanum* developed in sand flies arose from experimental studies in Panama (Shaw 1964). Arias et al. (1985) found that a total of 13.37% of sand flies of the Shannoni group collected at the base of the trees in the states of Acre, Amazonas (north and south of the Amazon River), Rondônia, and Pará had *Endotrypanum* infections. Their isozyme profiles showed that 6.29% of the isolates, all from Rondônia, belonged to 2 distinct groups. One, consisting of 16 isolates, was considered as *E. schaudinni*, and the other, composed of 8 strains, was called *Endotrypanum* species. The first group was classified as *E. schaudinni* because its iso-enzymatic profile was identical to that of a sloth isolate from the Jari region of Pará, except for the mobility of enzyme MPI, which was slightly faster. However, the 2 groups showed differences in 7 enzymatic loci. Some of the infections identified as *E. schaudinni* were associated with blood-meal remnants (Arias and Naiff personal communication).

The results of experimental infections of Brazilian phlebotomines with parasites identified as *Endotrypanum* are controversial. Shaw (1981) successfully infected laboratory-reared *Bi. flaviscutellata*, *Lu. longipalpis* (Marajó), and *Vi. furcata* fed on *Endotrypanum*-infected *Choloepus didactylus* sloths. The infections were like those described by the same investigator in 1964 in Panamanian phlebotomines. However, the investigator did not succeed in infecting another group of *Lu. longipalpis* from the same colony with *Endotrypanum* cultures. The developmental cycle in the pylorus region was like that of *Leishmania* of the subgenus *L.* (*Viannia*), but the invasion of Malpighian tubules was also observed, which does not occur in peripylarian *Leishmania*. This is characteristic of other sand-fly trypanosomatids, excluding *Leishmania*, and clearly distinguishes *Endotrypanum* infections from those caused by *Leishmania* of the subgenus *L.* (*Viannia*), although both develop as promastigotes. However, others (Franco et al. 1997; Barbosa et al. 2006) failed to see this, but their infections were initiated with culture and not blood forms. One possible explanation is that parasites that tolerate or prefer acid pH levels are lost during cultivation in neutral or alkaline media.

Franco et al. (1997) experimentally infected laboratory-bred *Ps. shannoni* originating from the United States, sand *Lu. longipalpis* from Lapinha cave, Brazil, and Colombia with strains of *Endotrypanum* that had been isolated (Arias et al. 1985) from a *Ps. shannoni* and a two-toed sloth captured in Rondônia. Parasites isolated from flies of the Shannoni series infected 100% of *Ps. shannoni* but only 62.3% of *Lu. longipalpis*. The Colombian lineage of *Lu. longipalpis* was significantly less infected with a sloth strain than that of the Shannoni group (18.2% compared with 55.6%). According to the reference number of the strains, it is probable that the parasites of the Shannoni series and those of sloths originate in different regions of the Amazon. In another set of experiments, Barbosa et al. (2006) found that *L. (V.) guyanensis* inhibited infections of *Endotrypanum* in a Lapinha-cave *Lu. longipalpis* colony.

What could be the reason for the contrasting results of the experimental infections of *Lu. longipalpis* with *Endotrypanum* cultures? In one case (Shaw 1981), no infections were obtained; however, in the others (Franco et al. 1997; Barbosa et al. 2006) they were. One explanation could be in the different origins of the *Lu. longipalpis* colonies. The ones that were negative originated from Marajó, Pará, whereas those that were positive came from the Lapinha Cave, Minas Gerais. The Marajó population has a burst-type mating song and cembrine pheromone, and the Lapinha cave population has a pulse-type 2 mating song and a 9-methylgermacrene-B pheromone. They are therefore different sibling species that could have different susceptibilities, which would explain the completely opposite results. What adds weight to this is that the positive infections were in two different laboratories but with Lapinha-cave *Lu. longipalpis* colonies. A problem with this hypothesis is that blood forms did infect Marajó *Lu. longipalpis.* Further evidence for parasite/vector specificity of flagellates identified as *Endotrypanum* is needed to explain these contradicting results.

Another complex question is the identity of parasites considered to be *Endotrypanum.* Arias et al. (1985) appears to have been justified in placing the parasites for sand flies of the Shannoni series in different taxa. However, the question is whether it was correct to use the name *Endotrypanum* for the group of parasites of that had very isoenzymatic profiles from *E. schaudinni*. Franco et al. (1996) identified 12 *Endotrypanum* zymodemes amongst isolates from sloths, but there was no association between their geographical origins. A more recent molecular study (Espinosa et al. 2016) confirmed high levels of genetic variability and two well-supported clades. Again, there was no clear geographical association with the clades and the geographical origin of their strains, but one was composed of strains from the States of Pará and Amazonas.

There is evidence that Rondônia is in a region that constitutes a biogeographic bridge between east and west (Patton and da Silva 1998). Thus, it is possible that two distinct *Endotrypanum* taxa could coexist in this region. It is an attractive hypothesis; however, it does not explain why *E. schaudinni* isolates of sand flies infected a consistently higher percentage of sand flies reaching 100% in the homologous *Ps. shannoni*. The taxonomic position of sand-fly infections considered to be *Endotrypanum* will only be clearer when a greater number of strains are examined and compared with those from mammals.

Concluding Remarks

This review shows that Brazilian sand flies are hosts to hundreds of microorganisms in addition to those belonging to the different *Leishmania* subgenera. These insects clearly represent a potential meeting point for many different parasites. However, more studies are needed before it can be said that they are in fact vectors of these organisms. A crucial question is this: Can one affect the development of the other? Because viruses are so common in sand flies, it is questioned whether or not they would affect the development of trypanosomatids in the vector organism.

The variety of virus found in sand flies is astounding, and there is evidence of transovarian transmission. The surprising thing is that these viruses are only occasionally found in vertebrate hosts. Pacui was isolated 100 times from pools of *Bi. flaviscutellata* captured in the Utinga forest near Belém (Aitken et al. 1975), and 30% of the wild rodents studied (including two from Amapá) and 10% of the marsupials from the same locality were serologically positive. These findings reinforce the thesis that studies carried out during periods of high transmission are more likely to elucidate the vector/reservoir cycle.

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